ENHANCED PROSTAGLANDIN I₂-FORMATION OF HUMAN LYMPHATICS DURING PULSATILE PERFUSION

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ABSTRACT

Previous studies demonstrate that prostacyclin (prostaglandin I₂, PGI₂) is the main arachidonic acid product in human lymph vessels. Pulsatile perfusion increases and prolongs PG₁2-formation as do leukotrienes (LT) such as LTC₄. Thus, physical activity besides local mechanical and biochemical influences on lymph pressure and flow also stimulates local lymphatic PG₁2 synthesis, a prime counterbalancing factor in lymphatic constriction induced by other eicosanoids.

Human lymphatics like arteries (1) and veins (2) produce notable amounts of prostaglandin (PG) (3). The in vitro PG₁2-synthesis (4), however, as well as the conversion rate (5) in the presence of exogenously added [³H]-arachidonic acid (AA) does not reflect the actual amount of biologically active PG₁2 at the site of its action. Thus, leukotrienes (LT) C₄ and D₄ are both capable of enhancing the PG₁2-generation on a dose-dependent basis (6). This phenomenon, for example, may play an important role during inflammation where white blood cells provide LT's (7) in excess at the inflamed site. Enhanced eicosanoid formation induced by cellular damage (8), hypoxia (9) and mechanical irritation (10) are other factors promoting release of contractile stimulants, such as thromboxane A₂ (11), and LT's (12) eicosanoids that offset the vasorelaxant effect of PG₁2 (13). Increased intraluminal pressure as with exercise has also been suggested to stimulate PG₁2-formation in blood vessels (14).

Because the intraluminal pressure of in vivo lymphatics varies widely during physical activity we examined whether graded increments in lymphatic pressure altered formation of prostaglandin I₂ in vitro.

MATERIALS AND METHODS

We examined four human peripheral lymphatics from three females and one male (age 15-42 years). Lymphatics were stored in liquid nitrogen (-70°C) until testing. A control segment of the lymph vessel was incubated in the perfusion system (Fig. 1) but without perfusion. The incubation buffer (tris-HCl, pH 7.4) was removed every ten minutes for 120 minutes and determined promptly for the presence of prostaglandin I₂ using the platelet aggregation bioassay (16). Briefly, 100 µl of the supernatant was added to an aggregometer one minute prior to induction of aggregation by 1 µM (100 µl) ADP. The aggregation inhibition was quantified using a synthetic PG₁2-standard. The characteristics of the platelet aggregation inhibitory compound were classified as being PG₁2 as described earlier (7). The amount of PG₁2 is given in pg PG₁2 per cm² per minute.
Perfusion experiments

Lymph vessels were perfused under pulsatile flow at graded pressures of 30/60, 60/120 and 80/120 mmHg with 60 pulsations per minute. 0.15 ml/min solution was perfused and the effluent was collected in an ice-bath as shown in Fig. 1. As with control lymphatics, incubated fluid was collected serially for 120 minutes and the amount of prostacyclin in the effluent tested using the bioassay technique, and the half-life of PGI₂-formation was computed.

Statistics

The values are given in X ± SD; a comparison has been done using Student’s t-test for assessing statistical significance.

RESULTS

Perfusion with pulsatile pressure in vitro significantly increased and prolonged prostaglandin I₂-formation. The increase in prostacyclin formation was dose-dependent (Table 1); however, the half-life of PGI₂-generation was unchanged (22 ± 4, 24 ± 3 and 21 ± 4 min., respectively). The addition of leukotriene C₄ (50 ng/ml) induced a further increase in PGI₂-formation, but again the half-life of PGI₂ was unchanged (25 ± 4 min.).

DISCUSSION

Intralymphatic pressure varies considerably depending on physical activity, (e.g., walking, running, sitting, recumbency). Our findings demonstrate, that in an isolated human lymphatic, PGI₂-formation is definitely pressure dependent. Nonetheless, it should be noted, that this lymph vessel is removed from its normal physiological environment including neurohumoral regulating controls. Because prostaglandin I₂ relaxes precontracted lymphatics and antagonizes vasoconstriction (13), the amount of biologically active PGI₂ available at a local site may be important for overall modulation of lymphatic tone. Unfortunately, the active local concentrations of the contractile agents (thromboxane A₂, prostaglandin G₂, H₂, leukotrienes) are difficult if not impossible to assess in vivo. Thus, it is still speculative whether this pressure-dependent response of prostaglandin I₂-generation operates in vivo. It is also noteworthy that clinical conditions with increased lymph flow are associated with increased formation of either thromboxane or leukotrienes (e.g. endotoxemia (20) or local inflammation (6)). Thus, lymph flow with physical movement (21) is not only propelled by mechanical forces, but also by the prostaglandin system. Other environmental
Table 1.
Prostaglandin I₂-formation (cm²/min.)
With Increasing Pulsatile Pressure (x ± SD)

<table>
<thead>
<tr>
<th>Min.</th>
<th>Static</th>
<th>30/20 mmHg</th>
<th>60/40 mmHg</th>
<th>120/80 mmHg</th>
<th>60/40 mmHg +50ng LTC₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>3.62 ± 1.67</td>
<td>6.12 ± 1.56</td>
<td>11.26 ± 2.17</td>
<td>28.41 ± 5.21</td>
<td>27.24 ± 7.63</td>
</tr>
<tr>
<td>20</td>
<td>1.86 ± 0.61</td>
<td>3.12 ± 1.23</td>
<td>8.24 ± 1.63</td>
<td>21.54 ± 4.17</td>
<td>16.32 ± 6.56</td>
</tr>
<tr>
<td>30</td>
<td>1.08 ± 0.37</td>
<td>2.27 ± 0.64</td>
<td>5.26 ± 1.47</td>
<td>17.33 ± 3.16</td>
<td>10.54 ± 5.21</td>
</tr>
<tr>
<td>40</td>
<td>0.26 ± 0.11</td>
<td>1.13 ± 0.71</td>
<td>4.55 ± 1.16</td>
<td>11.62 ± 2.84</td>
<td>8.75 ± 3.63</td>
</tr>
<tr>
<td>50</td>
<td>0.09 ± 0.05</td>
<td>1.47 ± 0.41</td>
<td>2.75 ± 0.56</td>
<td>8.55 ± 3.06</td>
<td>8.54 ± 2.86</td>
</tr>
<tr>
<td>60</td>
<td></td>
<td>1.33 ± 0.36</td>
<td>1.82 ± 0.63</td>
<td>6.36 ± 1.86</td>
<td>8.64 ± 2.73</td>
</tr>
<tr>
<td>70</td>
<td></td>
<td>1.55 ± 0.30</td>
<td>1.85 ± 0.41</td>
<td>3.71 ± 1.24</td>
<td>4.13 ± 1.56</td>
</tr>
<tr>
<td>80</td>
<td></td>
<td>0.83 ± 0.41</td>
<td>1.77 ± 0.21</td>
<td>4.22 ± 0.86</td>
<td>5.34 ± 1.85</td>
</tr>
<tr>
<td>90</td>
<td></td>
<td>0.74 ± 0.27</td>
<td>1.93 ± 0.56</td>
<td>2.13 ± 0.71</td>
<td>4.13 ± 1.21</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td>0.26 ± 0.09</td>
<td>0.84 ± 0.21</td>
<td>2.34 ± 1.12</td>
<td>2.65 ± 0.86</td>
</tr>
<tr>
<td>110</td>
<td></td>
<td>0.37 ± 0.12</td>
<td>0.66 ± 0.33</td>
<td>1.11 ± 0.26</td>
<td>2.24 ± 0.57</td>
</tr>
<tr>
<td>120</td>
<td></td>
<td>0.22 ± 0.08</td>
<td>0.21 ± 0.08</td>
<td>0.63 ± 0.31</td>
<td>1.23 ± 0.48</td>
</tr>
</tbody>
</table>

Factors that regulate the biological half-life of PGI₂, such as protein concentration and pH, also contribute to the complexity of local lymph propulsion. On the other hand, observations that upstream obstruction of lymphatics (22) promotes a decrease in frequency of spontaneous contraction while downstream obstruction increases lymphatic contractility are still not readily explained. Although the in vitro findings favor a pressure-dependent increase in prostacyclin generation, the role of pulsatile flow and PGI₂-synthesis is still controversial (15,19). Nonetheless, greater lymphatic tone probably increases lymphatic pulsatility and therefore accelerates forward lymph flow and in this setting local PGI₂-production may act as a sensitive feedback control mechanism to regulate human lymphatic motility.

REFERENCES


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